Fundus autofluorescence: Applications and perspectives

J. Cuba *, F. Gómez-Ulla

Servicio de Oftalmología, Hospital Médico-Quirúrgico de Conxo, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain

ABSTRACT

Purpose: To describe the findings of the study of autofluorescence of the different retinal diseases included in the study. To determine in which diseases autofluorescence may be more, or just as, useful as fluorescein angiography (FAG) in terms of diagnostic information.

Material and methods: We studied the retinal autofluorescence of 123 eyes of 93 patients, including various diseases of the eye fundus. In all cases we explored the fundus, retinal autofluorescence, and, if indicated, FAG was performed. Analysis of the autofluorescence was performed using the Heidelberg Retina Angiograph 2 (HRA2) Heidelberg Engineering (Germany).

Results: The autofluorescence information provided was equal or better (than FAG) in: 68.18% of cases of macular edema, 50% of pigment epithelium detachments, 100% of pigment epithelium atrophies, 100% of central serous chorioretinopathy; 55.55% of choroidal neovascularization, 100% of retinal dystrophies with deposition of lipofuscin, 100% of hard exudates and pre-retinal hemorrhages.

Conclusions: Autofluorescence is a quick and non-invasive examination method, comfortable for both patient and examiner, and with a very short learning curve. It provides diagnostic information about many eye fundus diseases. While more studies and more experience with its use are needed, its interest lies in the possibility of avoiding the performing of angiography in patients with these diseases, and in the additional information autofluorescence provides about the functional situation of cells and retinal pigments.

© 2011 Sociedad Española de Oftalmología. Published by Elsevier España, S.L. All rights reserved.

Palabras clave: Autofluorescence retiniana Autofluorescence of the eye fundus Lipofuscin

Objetivos: Describir los hallazgos en el estudio de la autofluorescencia de las distintas enfermedades retinianas incluidas en el estudio. Estudiar en qué enfermedades la autofluorescencia puede ser igual o mejor que la angiografía fluoresceínica (AGF) cuan a información diagnóstica.

* Please cite this article as: Cuba J, Gómez-Ulla F. Autofluorescencia retiniana: aplicaciones y perspectivas. Arch Soc Esp Oftalmol. 2013;88:50–5.

** Partially presented as a panel communication at the 83rd Congress of the Spanish Ophthalmological Society, Las Palmas de Gran Canaria, Spain. 1st Prize in the panel category.

* Corresponding author.

E-mail address: judit.cuba@gmail.com (J. Cuba).

2173-5794/$ – see front matter © 2011 Sociedad Española de Oftalmología. Published by Elsevier España, S.L. All rights reserved.
Material y métodos: Se estudió la autofluorescencia retiniana de 123 ojos y pacientes, incluyendo diversas enfermedades del fondo de ojo. En todos los casos se exploró el fondo de ojo, la autofluorescencia retiniana, y, en caso de estar indicado, se realizó AGF. La exploración de la autofluorescencia fue llevada a cabo usando el angiógrafo Heidelberg Retina Angiograph 2 (HRA2) de Heidelberg Engeneering (Alemania).

Resultados: La autofluorescencia aportó una información igual o mejor (que la AGF) en: 68,18% de casos de edema macular; 50% de desprendimientos del epitelio pigmentario; 100% de atrofas del epitelio pigmentario; 100% de coriorretinopatías centrales serosas; 55,55% de neovascularizaciones coroideas; 100% de las distrofas retinianas con depósito de lipofuscin; 100% de los exudados duros y hemorragias preretinianas.

Conclusiones: La autofluorescencia es un método de exploración rápido, cómodo para el paciente y explorador, no invasivo y con una curva de aprendizaje muy corta, que aporta información para el diagnóstico de múltiples enfermedades con afectación del fondo de ojo. Si bien son precisos más estudios y más experiencia con su uso, su interés radica en la posibilidad de evitar la realización de angiografías en pacientes con estas enfermedades y en la información adicional que nos aporta sobre la situación funcional de las células y pigmentos retinianos.

© 2011 Sociedad Española de Oftalmología. Publicado por Elsevier España, S.L. Todos los derechos reservados.

Introduction

Ocular fundus autofluorescence (AF) is an intrinsic capacity exhibited by the normal human retina consisting in the emission of light in the wavelength range of 500-700 nm when excited by a short wavelength light between 470 and 500 nm. The main autofluorescent component of the eye fundus is lipofuscin that, under normal conditions, forms part of the retina pigment epithelium (RPE). Therefore, changes in the RPE such as abnormal lipofuscin deposits or atrophy will bring about changes in AF. However, other components may contribute to or affect the AF signal. Yellow macular pigments such as lutein or zeaxanthin attenuate the blue light used to record AF, and their distribution in the ocular fundus renders AF lower in the macular area and even lower in the fovea. It has also been described that the photopigment of cones as well as changes in retinal metabolic rates could affect AF, although it seems sensible to assume the existence of components still unknown to us.1-4

The main objective of this paper is to describe the findings observed in studying AF in various retinal diseases included in the paper. It also includes the practical hypotheses that AF could be better or at least equivalent to fluorescein angiography (FAG) to provide diagnostic information in some ocular fundus diseases.

Subjects, material and methods

The AF of 123 eyes of 93 patients was studied, including the following groups:

1. 18 eyes of 13 patients having eye fundus without pathological alterations.
2. 29 eyes of 22 patients with macular edema.
3. 6 eyes of 5 patients with pigment epithelium detachment (PED).
4. 28 eyes of 21 patients with RPE atrophy of various etiologies:
   i. 5 eyes of 3 patients with age-related atrophic maculopathy.
   ii. 13 eyes of 11 patients with RPE atrophy secondary to retinal laser photocoagulation.
   iii. 6 eyes of 4 patients with RPE peripapillary atrophy.
   iv. 5 eyes of 4 patients with other types of RPE atrophy (one of the patients exhibited in the same eye RPE peripapillary atrophy as well as atrophy secondary to laser photocoagulation and for this reason said eye was included in 2 subgroups for the statistical analysis).
5. 3 eyes of 3 patients with central serous chorioretinopathy (CSC).
6. 9 eyes of 9 patients with choroidal neovascularization (CNV):
   i. 2 eyes of 2 patients with CNV not associated to age-related macular degeneration (ARMD).
   ii. 7 eyes of 7 patients with CNV associated to ARMD.
7. 11 eyes of 6 patients with retinal dystrophies subdivided in:
   i. Concentric to the macula:
      a. 6 eyes of 3 patients with Stargardt disease.
      b. 2 eyes of 1 patient with Best disease.
      c. 1 eye of 1 patient with pattern (butterfly wing) macular dystrophy.
   ii. Peripheral:
       a. 2 eyes of 1 patient with pigmentary retinosis.
8. 3 eyes of 3 patients with papilledema.
9. 16 eyes of 11 patients with other ocular fundus alterations.
   i. 3 eyes of 3 patients with drusen.
   ii. 4 eyes of 3 patients with hard exudates secondary to diabetic macular edema.
   iii. 6 eyes of 6 patients with preretinal hemorrhage.
   iv. 4 eyes of 2 patients with glaucoma (three of the patients concurrently exhibited hard exudates and preretinal hemorrhage, one of them in the same eye. These eyes and patients were counted in both subgroups for the statistical analysis).
The patients whose ocular fundus was difficult to visualize for any reason such as pupil dilatation under 6 mm after administrative mydriatic eyedrops, presence of leukemia, tyndall, flare, lens opacities, hemovitreous or any other cause of opacity in transparent ocular media were excluded. In addition, the patients who were unable to fix their gaze for exploration or exhibited intolerance to fluorescein were also excluded.

The AF exploration was carried out in all cases with the Heidelberg Retina Angiograph 2 (HRA2) by Heidelberg Engineering (Heidelberg, Germany). This device is a confocal laser scanning system designed for studying the retina and the various wavelengths as well as for performing FAG or with indocyanine green. The exciting wavelength used for the study of AF is 488 nm, and the light reflected by the retina passes through a scanned filter that allows passage at a wavelength over 500 nm.

In addition, in all cases indirect funduscopy was carried out with non-contact lenses of 78 diopters or similar, and in 80 eyes digital color retinography was performed (using the TOPCON TRC-50IX retinal camera angiograph, TOPCON, Japan). FAG was done when indicated and that was the case in 82 eyes. Both the HRA2 and the TOPCON TRC-50IX were utilized for this purpose.

Supplementary tests were also made when necessary to reach the certainty diagnostic.

When the certainty diagnostic was available, the AF and FAG images were analyzed, classifying the alterations according to their intensity in hypo- or hyperfluorescence.

Finally, the ability of each exploration (AF, FAG and funduscopy) to define an alteration in the ocular fundus was expressed qualitatively by means of the following symbols:

+: poor definition.
++: intermediate.
++++: good.
+++++: excellent.

Results

Normal ocular fundus (18 eyes). In all cases (100%) the AF took the pattern shape considered to be normal that was described in the introduction of this paper (Fig. 1).

Macular edema (29 eyes). In 100% of cases, the accumulation of liquid at the macular level was expressed hyperfluorescent in AF as well as in FAG. The capacity exhibited by AF to define the edema area is worthy of note. AF made a better definition of the lesion in 4.54% of cases while FAG did so in 31.82% of cases. In the remaining 63.64% of cases the definition made by both tests was equivalent (Fig. 2).

PED (6 eyes). Both AF as well as FAG exhibited hyperfluorescence of the lesion in 85.71% and 75% of cases respectively. AF made a better definition of half of cases. FAG was superior in the other half.

RPE atrophy (28 eyes). The atrophy was shown as hypo-fluorescent in all the AF images and hyperfluorescent in all the FAG images. The definition of the atrophy area made by both tests was similar in all cases.

CSC (3 eyes). All the cases exhibited hypo-fluorescence in AF while in the angiographic behavior was more heterogeneous with one case exhibiting hyperfluorescence, another one hypo-fluorescence and a third case iso-fluorescence. In all cases, AF made a better definition of the lesion. In addition, only one case demonstrated the existence of a leak point, which was visible both in FAG as well as in AF (Fig. 3).

CNV (9 eyes): in our patients both AF as well as FAG exhibited predominantly hypo-fluorescent lesions in 77.78% and 66.67% of cases respectively. FAG made a better definition of the lesion in a 44.44% of cases (Fig. 4).

Retinal dystrophies

Concentric to the macula (9 eyes): the abnormal deposit of lipofuscin was shown as hyperfluorescence in all the AF images and hypo-fluorescent in all the angiographies. In contrast, the secondary RPE atrophy was hypo-fluorescent in 100% of AF and hyperfluorescent in 100% of FAG. The definition provided by AF was better (22.22%) or the same (77.78%) as FAG in all cases.

Pigmented retinositis (2 eyes): pigment in osteoclasts observable in the ocular fundus was expressed as hypo-fluorescent in AF.

Papilla edema (3 eyes): the AF image exhibited blurred papillary limits but no case exhibited changes in the natural hypo-autofluorescence of the optic nerve.

Other ocular fundus diseases

Drusen (3 eyes): all the drusen were hyperfluorescent in AF.
Fig. 2 – Diabetic cystic macular edema with focal photocoagulation in ocular fundus autofluorescence (left). Comparison with late angiography times (right).

Fig. 3 – Acute central serous chorioretinopathy in the posterior pole of the right eye, defining and showing the leak point both in autofluorescence (left) as in angiography (right).

Fig. 4 – Angiographic appearance (right) and in autofluorescence (left) of a predominantly classic choroidal neovascularization membrane.
Hard exudates (4 eyes): in all cases hard exudates performed as hyperfluorescent in AF and isofluorescent in FAG.

Preretinal hemorrhage (6 eyes): all of the lesions included in the study were hypo-fluorescent both in AF as well as in FAG.

Glaucoma (4 eyes): of the 4 eyes included in the study, only one exhibited treatment-resistant glaucoma, and only in this case an increase of the peri-papillary AF was evidenced. In the rest of cases no AF alterations were observed.

The results for the most relevant diseases included in the study are shown grouped and summarized in Table 1.

Discussion

Ocular fundus exploration with AF is an emerging technique which is not yet part of routine clinical practice. AF is still at the testing stage to prove what it can offer and to assess in which diseases it can be most useful.

An apparently obvious conclusion drawn from our results is that AF seems to be particularly useful for diagnosing diseases with the merely more for logical involvement of the RPE, in contrast with ocular fundus alterations involving a vascular/exudative (dynamic) component. Accordingly, RPE atrophy of any cause as well as abnormal accumulations of lipofuscin are very well defined with AF. This matches the good results obtained by the authors in the diagnostic study of geographical dry ARMD atrophy\(^1\)\(^-\)\(^3\) and macular dystrophies. In the pathogeny of these diseases, lipofuscin and atrophy play a fundamental role.\(^1\)\(^,\)\(^5\) The “negative” image of AF vis-à-vis typical FAG images of retinal dystrophies (Fig. 5) is because the lipofuscine pigments is shown as hyperfluorescent in AF and hypo-fluorescent in FAG (due to the locking effect of choroidal fluorescence secondary to the accumulation), while the opposite occurs in the case of APR atrophy which appears as hypo-fluorescent in AF but hyperfluorescent in FAG (due to the window effect that enables a view of choroidal fluorescence).

On the contrary, AF provides more heterogeneous and less consistent results when exploring vascular alterations such as CNV either secondary to ARMD\(^1\)\(^,\)\(^6\) or due to other causes. Even so, the fact that changes in AF can predict the evolution of exudative ARMD or have some prognostic value is a controversial subject.\(^7\) In addition, the good results offered by AF in the study of macular edema\(^1\)\(^,\)\(^8\) and

---

Fig. 5 – “Negative” images. Pseudovitelliform macular dystrophy (Best's disease) studied with autofluorescence (top, left) and angiography (top, right). Butterfly wing pattern macular dystrophy, autofluorescence (bottom, left), angiography (bottom, right).
Table 1 - Summary of results for each group in the most relevant diseases included in the study.

<table>
<thead>
<tr>
<th>n</th>
<th>Disease</th>
<th>AF</th>
<th>FAG</th>
<th>Best definition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypo</td>
<td>Hyper</td>
<td>Hypo</td>
<td>Hyper</td>
</tr>
<tr>
<td>29</td>
<td>Macular edema</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>PED</td>
<td>14.28</td>
<td>85.71</td>
<td>25</td>
</tr>
<tr>
<td>29</td>
<td>RPE atrophy</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>CSC</td>
<td>0</td>
<td>100</td>
<td>33.33</td>
</tr>
<tr>
<td>9</td>
<td>CNV</td>
<td>77.78</td>
<td>22.22</td>
<td>66.67</td>
</tr>
<tr>
<td>11</td>
<td>Dystrophies, atrophy</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Drusen</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Hard exudates</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Preterinal hemorrhages</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

In percentages.

a Percentage of lesions that were displayed as predominantly hypo-fluorescent.
b Percentage of lesions that were displayed as predominantly hyper-fluorescent.
c Percentage of lesions best defined by autofluorescence.
d Percentage of lesions in which the definition provided by angiography and autofluorescence was similar.
e Percentage of lesions best defined by angiography.

Central serous chorioretinopathy1,3,9 are worthy of note as the pathogenic of these diseases involve an exudative component. Some publications have affirmed that on its own AF would be able to detect leak points in central serous chorioretinopathy, although to date this is just another interesting line of research.5,9

In summary, AF is a fast, simple and comfortable exploration for the examiner as well as the patient, exhibiting a very short learning curve and the advantage over FAG that it does not require the injection of intravenous contrast in the bloodstream of the patient. This technology has given rise to major expectations and which, when analyzing its origins (including RPE and retina fluoropher cells and pigments), appears as a very valuable tool to assess and follow up the health, performance and possible deterioration of cells. And even though it appears to be superior to FAG for studying certain diseases, for the alterations in which FAG is superior to AF we should still consider the assistance of FAG to supplement the diagnostic and perform the follow-up. In this way we would avoid unnecessary repetition of invasive tests. On the other hand, it must be said that the limitation of AF has been already demonstrated in yellowish lenses or incipient cataracts.

It is clear that more experience is required, together with more patients in most studies including the diseases mentioned above as well as others so that in the next few years all the clinical applications of this promising technology for exploring the retina will be mastered and available.

Conflict of interest
No conflict of interest has been declared by the authors.

REFERENCES